

IN THE CLAIMS

1. (Previously Presented) A method of assessing the effect of a test condition on G-protein-coupled receptor (GPCR) pathway activity, comprising:

a) providing a cell that expresses a GPCR as a fusion protein to a first mutant form of a reporter enzyme and an arrestin as a fusion protein to a second mutant form of the reporter enzyme complementary to the first mutant form of the reporter enzyme,

wherein the arrestin is modified to enhance binding of said arrestin to the GPCR, wherein said enhanced binding between said arrestin and the GPCR increases sensitivity of detection of said effect of the test condition;

b) exposing the cell to a ligand for said GPCR under the test condition; and

c) monitoring activation of said GPCR by complementation of the first and second mutant forms of the reporter enzyme;

wherein increased reporter enzyme activity in the cell compared to that which occurs in the absence of the test condition indicates increased GPCR interaction with the modified arrestin compared to that which occurs in the absence of the test condition, and decreased reporter enzyme activity in the cell compared to that which occurs in the absence of the test condition indicates decreased GPCR interaction with the modified arrestin compared to that which occurs in the absence of the test condition; and

wherein the GPCR and the first mutant form of reporter enzyme are linked together by a polypeptide linker represented by the formula $-(GGGGS)_n-$ (SEQ ID NO:10).

2 - 8. (Canceled)

9. (Previously Presented) A method of assessing the effect of a test condition on G-protein-coupled receptor (GPCR) pathway activity, comprising:

a) providing a cell that expresses a GPCR as a fusion protein to a first mutant form of a reporter enzyme and an arrestin as a fusion protein to a second mutant form of the reporter enzyme complementary to the first mutant form of the reporter enzyme,

wherein the arrestin is modified by introducing a point mutation in a phosphorylation-recognition domain to remove a requirement for phosphorylation of the GPCR for arrestin binding to permit binding of the arrestin to said GPCR in the cell regardless of whether the GPCR is phosphorylated,

b) exposing the cell to a ligand for said GPCR under the test condition; and

c) monitoring activation of the GPCR by complementation of the first and second mutant forms of the reporter enzyme;

wherein increased reporter enzyme activity in the cell compared to that which occurs in the absence of the test condition indicates increased GPCR interaction with the modified arrestin compared to that which occurs in the absence of the test condition, and decreased reporter enzyme activity in the cell compared to that which occurs in the absence of the test condition indicates decreased GPCR interaction with the modified arrestin compared to that which occurs in the absence of the test condition; and

wherein the GPCR and the first mutant form of reporter enzyme are linked together by a polypeptide linker represented by the formula $-(GGGGS)_n-$ (SEQ ID NO:10).

10. (Previously Presented) The method of Claim 9, wherein the arrestin is mutated to increase a property selected from affinity and avidity for activated, non-phosphorylated GPCR.

11. (Previously Presented) The method of Claim 10, wherein the arrestin is β -arrestin2 and wherein the β -arrestin2 is mutated to convert Arg169 to an oppositely charged residue.

12. (Previously Presented) The method of Claim 11, wherein the oppositely charged residue is selected from the group consisting of histidine, tyrosine, phenylalanine and threonine.

13. (Previously Presented) The method of Claim 9, wherein the arrestin is mutated to increase a property selected from affinity and avidity for activated and phosphorylated GPCR.
14. (Canceled)
15. (Previously Presented) The method of Claim 1, wherein the modified arrestin exhibits enhanced binding to activated, phosphorylated GPCR.
16. (Previously Presented) The method of Claim 1, wherein the modified arrestin comprises conversion of Arg169 to an amino acid selected from the group consisting of histidine, tyrosine, phenylalanine and threonine.
17. (Previously Presented) The method of Claim 1, wherein the modified arrestin comprises conversion of Val170 to alanine.
18. (Previously Presented) The method of Claim 1, wherein the arrestin is selected from the group consisting of β -arrestin1 and β -arrestin2, and wherein the β -arrestin1 or the β -arrestin2 is truncated for all or part of a carboxyl-terminal half of the β -arrestin1 or the β -arrestin2.
19. (Previously Presented) The method of Claim 18, wherein the β -arrestin1 or the β -arrestin2 is truncated from amino acid 190 of the β -arrestin1 or the β -arrestin2 to the carboxyl-terminal end of the β -arrestin1 or the β -arrestin2.
20. (Previously Presented) The method of Claim 1, wherein the arrestin is a chimera of β -arrestin1, β -arrestin2 and/or visual arrestin.
21. (Previously Presented) The method of Claim 10, wherein the arrestin is a chimera of β -arrestin1, β -arrestin2 and/or visual arrestin.
22. (Previously Presented) The method of Claim 11, wherein the arrestin is a chimera of β -arrestin1, β -arrestin2 and/or visual arrestin.
23. (Previously Presented) The method of Claim 12, wherein the arrestin is a chimera of β -arrestin1, β -arrestin2 and/or visual arrestin.

24. (Previously Presented) The method of Claim 10, wherein the arrestin is β -arrestin2 and wherein the β -arrestin2 is mutated to convert Arg170 to an oppositely charged residue.

25. (Previously Presented) The method of Claim 1, wherein the modified arrestin comprises conversion of Arg170 to an amino acid selected from the group consisting of histidine, tyrosine, phenylalanine and threonine.

26. (Canceled)

27. (Previously Presented) The method of Claim 1, wherein n is 2 or more.

28. (Previously Presented) The method of Claim 1, wherein n is 4.

29. (Previously Presented) The method of Claim 1, wherein the second mutant form of the reporter enzyme is linked to the C-terminal of the arrestin.

30 - 51. (Canceled)

52. (Previously Presented) The method of Claim 9, wherein n is 2 or more.

53. (Previously Presented) The method of Claim 9, wherein n is 4.

54 - 60. (Canceled)